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Na^+/H^+ exchange inhibition prevents endothelial dysfunction after I/R injury

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[1]*Division of Cardiovascular Diseases, Department of Internal Medicine, Mayo Clinic and Foundation, Rochester, Minnesota 55905;* [2]*Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226;* [3]*Department of Cardiovascular Pharmacology, Merck, D-64271 Darmstadt, Germany*

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Gumina, Richard J., Jeannine Moore, Pierre Schelling, Norbert Beier, and Garrett J. Gross. Na^+/H^+ exchange inhibition prevents endothelial dysfunction after I/R injury. *Am J Physiol Heart Circ Physiol* 281: H1260–H1266, 2001.—Whereas inhibition of the Na^+/H^+ exchanger (NHE) has been demonstrated to reduce myocardial infarct size in response to ischemia-reperfusion injury, the ability of NHE inhibition to preserve endothelial cell function has not been examined. This study examined whether NHE inhibition could preserve endothelial cell function after 90 min of regional ischemia and 180 min of reperfusion and compared this inhibition with ischemic preconditioning (IPC). In a canine model either IPC, produced by one 5-min coronary artery occlusion (1 × 5′), or the specific NHE-1 inhibitor eniporide (EMD-96785, 3.0 mg/kg) was administered 15 min before a 90-min coronary artery occlusion followed by 3 h of reperfusion. Infarct size (IS) was determined by 2,3,5-triphenyl tetrazolium chloride staining and expressed as a percentage of the area-at-risk (IS/AAR). Endothelial cell function was assessed by measurement of coronary blood flow in response to intracoronary acetylcholine infusion at the end of reperfusion. Whereas neither control nor IPC-treated animals exhibited a significant reduction in IS/AAR or preservation of endothelial cell function, animals treated with the NHE inhibitor eniporide showed a marked reduction in IS/AAR and a significantly preserved endothelial cell function ($P < 0.05$). Thus NHE-1 inhibition is more efficacious than IPC at reducing IS/AAR and at preserving endothelial cell function in dogs.

endothelial cell dysfunction; sodium-hydrogen exchange; ischemia-reperfusion

CORONARY REPERFUSION by thrombolytic therapy and/or coronary angioplasty is the standard strategy in managing acute myocardial infarction. Whereas early reperfusion of ischemic myocardium leads to salvage of the jeopardized tissue, reperfusion itself may exacerbate the injury sustained during the ischemic period (9). Reperfusion leads to an inflammatory response that includes leukocyte adhesion to the coronary microvasculature (42) and vasculature dysfunction.

Ischemia-reperfusion injury attenuates endothelial cell function as measured by the response to various endothelial cell-mediated vasodilatory agents in large coronary arteries and in coronary microvessels. Whereas numerous studies demonstrated the efficacy of Na^+/H^+ exchange (NHE) inhibition at conferring myocardial protection, no reports have explored whether NHE inhibition has any beneficial effects on the coronary vasculature. Recent studies (17) by our laboratory demonstrate that NHE inhibition is more efficacious than ischemic preconditioning (IPC) at conferring myocardial protection against prolonged (90 min) ischemia followed by reperfusion. Whereas the protective effects of NHE inhibition on cardiomyocytes clearly has been demonstrated, to date, the effect of NHE inhibition on endothelial cell function has not been examined following ischemia-reperfusion injury. Thus the aim of this study was to evaluate whether NHE inhibition affords protection against ischemia-reperfusion injury of the coronary endothelium in the dog heart in vivo.

METHODS

Materials. To perform our studies, we used the selective NHE-1 isoform inhibitor eniporide [EMD-96785, 2-methyl-5-methylsulfonyl-1-(1-pyrrollyl)-benzoylguanidine] (3).

Ischemia-reperfusion protocol. A standard canine myocardial ischemia-reperfusion protocol that we have previously published in detail was employed (18). This protocol included isolation of a proximal portion of the left anterior descending (LAD) coronary artery distal to the first diagonal branch from surrounding tissue and placement of a calibrated electromagnetic flow probe (Statham SP 7515, Gould-Statham) around the vessel. The flowmeter (Statham 2202) was used to measure LAD blood flow. A mechanical occluder was placed distal to the flow probe so that there were no branches between the flow probe and the occluder. The occluder was used to set the flow probe to zero, to occlude the LAD and to reperfuse the myocardium.

Dogs were randomly assigned to one of three groups. All dogs were subjected to 90 min of LAD occlusion and 3 h of reperfusion. In *group 1* (control group), saline was infused

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intravenously for 15 min immediately before 90 min of LAD occlusion. In *group 2*, IPC was accomplished by 5 min of LAD occlusion followed by 10 min of reperfusion immediately before 90 min of LAD occlusion (1 × 5′ IPC). In *group 3*, eniporide was infused intravenously at 3.0 mg/kg for 15 min immediately before 90 min of LAD occlusion. In all groups, hemodynamic measurements and blood gas analyses were conducted before LAD occlusion, at 30 min during LAD occlusion, and every hour after reperfusion. Regional myocardial blood flows were determined at 30 min during the LAD occlusion period and at the end of the experiment.

At the end of the 3-h reperfusion period, following measurement of coronary blood flow in response to acetylcholine, the anatomic area-at-risk (AAR) and the nonischemic area were differentiated, and the hearts were processed and stained via 2,3,5-triphenyltetrazolium chloride (TTC) to differentiate the infarcted myocardium as previously described (18). Infarct size (IS) was expressed as a percentage of the AAR. Regional myocardial blood flow was measured by the radioactive microsphere technique as described previously by Gross et al. (15).

Dogs were excluded if *1)* heartworms were found after the dogs were killed, *2)* transmural collateral blood flow was >0.20 ml·min^{-1}·g^{-1}, *3)* heart rate was >180 beats/min at the beginning of the experiment, or *4)* more than three consecutive attempts were needed to convert ventricular fibrillation with low-energy DC pulses applied directly to the heart.

Endothelial cell function. At the end of 3 h of reperfusion, the LAD was cannulated with a 22-gauge needle and coronary blood flow was allowed to stabilize. Acetylcholine at increasing concentrations was then infused, and the coronary blood flow was measured using the calibrated electromagnetic flow probe. Responses to the various doses of acetylcholine were allowed to reach a steady state before infusion of the subsequent dose.

Creatinine kinase activity and troponin I measurements. Blood samples were drawn from individual animals at preset time intervals. Samples were anticoagulated with EDTA and spun at 200 *g* to remove erythrocytes, and the plasma was stored at −20°C until assayed for creatinine kinase (CK) activity and troponin I. For the determination of CK activity, the Merck test kits Granutest 25 CK NAC, Granutest CK/CK-MB NAC Starter, and Granutest CK/CK-MB NAC Buffer were used. For determination of the plasma troponin I levels, the Troponin I Pasteur Kit (Sanofi Diagnostics Pasteur) was used according to the manufacturer's recommendations. Optical density was measured using a TECAN Spectra FLUOR microtiter plate reader. The cross species reactivity and specificity of the Sanofi Troponin I Pasteur Kit was determined using purified canine troponin I. Data are expressed as the mean of the area under the curve for CK activity and troponin I levels from 5 min before reperfusion through the entire 3-h reperfusion period.

Statistical analysis. All values are expressed as means ± SE. Differences between groups in hemodynamics and blood gases were compared with the use of a two-way (for time and treatment) ANOVA with repeated measures. Differences between groups in tissue blood flows, AAR, and IS were compared by one-way ANOVA and comparisons between individual groups were made with a two-tailed *t*-test. ANCOVA was used to determine whether the relationship between transmural collateral blood flow and IS differed between the control and treated groups. For all experiments differences between groups were considered significant if the probability value was $P < 0.05$. For examination of the release of CK and troponin I, the mean area under the curve was determined from the time point 5 min before reperfusion through the entire 3-h reperfusion period. The area-under-the-curve group values were compared using Duncan's multiple range test. The differences between the groups were considered significant ($P < 0.5$) if the observed statistic was greater than the critical value of the *q*-statistics.

RESULTS

Exclusions to canine ischemia-reperfusion studies. Twenty-four animals were used in this study. No animals were excluded; thus 24 dogs successfully completed the protocol and were included in data analysis.

Hemodynamic, blood gas data, and pharmacological profile. Heart rate, mean aortic blood pressure, the rate-pressure product, and left ventricular pressure development over time (dP/*dt*) data are summarized in Table 1. The baseline values were not different be-

Table 1. *Hemodynamic data*

	Baseline	Drug (15 min)	Occlusion (30 min)	Reperfusion 1 h	Reperfusion 2 h	Reperfusion 3 h
HR, beats/min						
Control	156 ± 2	155 ± 2	153 ± 4	152 ± 4	158 ± 3	157 ± 4
Eniporide	151 ± 2	153 ± 4	149 ± 4	149 ± 2	151 ± 2	153 ± 2
IPC (1 × 5′)	153 ± 5	154 ± 5	157 ± 3	157 ± 3	158 ± 3	159 ± 3
MBP, mmHg						
Control	98 ± 4	99 ± 6	90 ± 4	96 ± 5	98 ± 6	95 ± 7
Eniporide	114 ± 6	128 ± 6*	111 ± 5*	107 ± 5	117 ± 5	118 ± 5*
IPC (1 × 5′)	107 ± 7	103 ± 7	108 ± 7*	113 ± 5	115 ± 6	122 ± 6*
RPP, mmHg·min·1,000^{-1}						
Control	17.2 ± 0.8	17.3 ± 0.9	16.3 ± 0.8	16.4 ± 1.0	17.3 ± 1.3	17.2 ± 0.7
Eniporide	18.6 ± 1.1	20.6 ± 1.4	20.0 ± 1.1*	18.1 ± 1.0	18.2 ± 0.8	18.2 ± 0.8
IPC (1 × 5′)	19.0 ± 1.1	19.3 ± 1.3	19.7 ± 1.2*	18.7 ± 0.8	19.6 ± 0.9	19.8 ± 1.2
LV dP/*dt*, mmHg/s						
Control	1,867 ± 123	1,900 ± 130	1,625 ± 125	1,683 ± 86	1,700 ± 97	1,733 ± 133
Eniporide	2,014 ± 134	2,400 ± 160	2,036 ± 97	1,736 ± 79	1,886 ± 72	1,843 ± 102
IPC (1 × 5′)	1,988 ± 183	1,725 ± 177	2,100 ± 172	1,969 ± 100	1,875 ± 94	1,894 ± 127

All values are means ± SE; n = 7–9. HR, heart rate; MBP, mean aortic blood pressure; RPP, rate-pressure product; LV dP/*dt*, left ventricular (LV) pressure development over time; IPC, ischemic preconditioning. *$P < 0.05$ vs. control group by ANOVA.

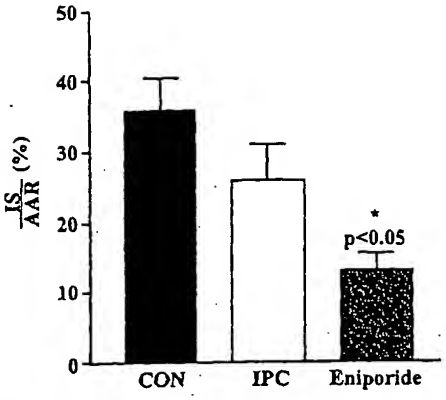

Fig. 1. Effect of Na^+/H^+ exchange (NHE)-1 inhibition on infarct size (IS). Ischemic preconditioning (IPC) (1 × 5′), eniporide (3.0 mg/kg), or saline (CON) was administered 15 min before occlusion of the left anterior descending (LAD) coronary artery for 90 min. IS versus the area-at-risk (AAR) for infarction (IS/AAR) is expressed as a percent. All values are means ± SE. n = 9 (CON), 8 (IPC), and 7 (eniporide) dogs. *P < 0.05 vs. control group.

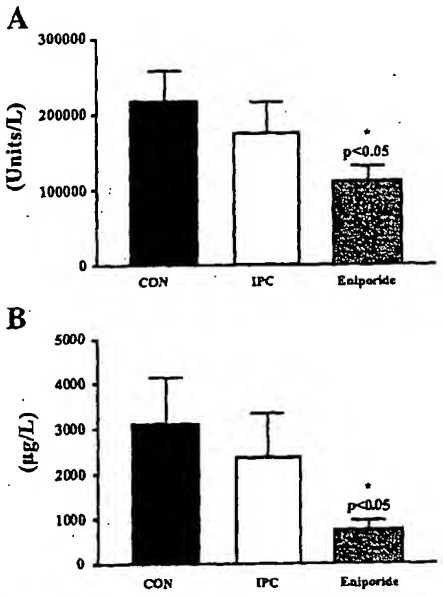

Fig. 2. Effect of NHE-1 inhibition on serum marker for myocardial ischemia. IPC (1 × 5′), or eniporide (3.0 mg/kg), or saline (CON) was administered 15 min before occlusion of LAD for 90 min. Data are expressed as mean value of the area under the curve for creatinine kinase (*A*) activity and troponin I (*B*) levels released from 5 min before reperfusion through the entire 3-h reperfusion period. n = 9 (CON), 8 (IPC), and 7 (eniporide) dogs. *P < 0.05 vs. control group.

tween groups; however, after administration of eniporide, there was a transient increase in blood pressure that returned to baseline at 30 min of coronary artery occlusion. Mean blood pressure was also higher in the IPC group compared with the control group at 30 min of occlusion and at 3 h of reperfusion, although these values were not significantly higher than the pre-IPC values determined at baseline. The rate-pressure product in the eniporide-treated and IPC-treated groups was higher at 30 min of occlusion compared with that of the corresponding control group. There were no differences in blood pH, P_{CO_2}, and P_{O_2} among groups at any time throughout the studies. The average maximal concentration of eniporide observed was 8.1 μM at 5 min postinfusion with an exponential first-order decay. A calculated half-time ($t_{1/2}$) of 1.2 h was determined from measurements conducted in this experimental protocol (data not shown).

Infarct size measurements. Figure 1 and Table 2 demonstrate the effect of IPC or NHE-1 inhibition on the AAR and IS determined by TTC staining and expressed as a percentage of the AAR (IS/AAR) and left ventricle (IS/LV). No statistically significant reduction in IS/AAR was observed with IPC (1 × 5′) (Fig. 1 and Table 2). In contrast, administration of 3.0 mg/kg of eniporide significantly reduced IS/AAR (P < 0.05).

There were no significant differences in LV weight, AAR, AAR/LV, or transmural collateral blood flow between treatment groups (Table 2). The regression lines describing the relationship between transmural collateral blood flows and IS/AAR were not different between the control and IPC groups. However, in animals treated with 3.0 mg/kg eniporide, the regression lines describing this relationship were significantly shifted down compared with the control and IPC-treated groups (data not shown).

Serum marker analysis. Examination of the release of CK activity and troponin I into the serum after ischemia and reperfusion injury demonstrated a significant decrease in levels of both in animals treated with eniporide (50% and 75%, respectively) compared with control and IPC-treated animals (Fig. 2).

Endothelial cell function. Acetylcholine has been shown to be an endothelium-dependent vasodilator in several isolated vessel preparations, as well as in the intact coronary circulation of the dog. After 90 min of ischemia and 3 h of reperfusion, IPC failed to significantly preserve coronary endothelial cell function as assessed by the lack of an increase in coronary blood flow in response to acetylcholine administration. In

Table 2. *Gravimetric and collateral flow data*

Group	n	Weight, g		Calculated Data, %				Transmural Blood Flow 30 min Occ, ml·min^{-1}·g^{-1}
		LV	AAR	IS	AAR/LV	IS/AAR	IS/LV	
Control	9	106.6 ± 3.7	35.3 ± 1.7	13.0 ± 2.0	33.2 ± 1.4	35.8 ± 4.4	12.2 ± 1.8	0.05 ± 0.02
Eniporide	7	96.9 ± 4.8	33.3 ± 3.8	4.5 ± 1.0*	33.9 ± 2.3	13.2 ± 2.3*	4.5 ± 0.9*	0.09 ± 0.02
IPC	8	107.5 ± 4.3	38.4 ± 2.6	10.4 ± 2.3	36.2 ± 3.1	26.1 ± 4.9	9.7 ± 2.5	0.08 ± 0.02

All values are means ± SE; n, number of dogs. AAR, area-at-risk; IS, infarct size; Occ, occlusion. *P < 0.05 vs. control group by ANOVA.

ischemia-reperfused myocardium (16). Thus attenuation of neutrophil activation and subsequent interactions with the endothelium may be responsible, in part, for the preservation of endothelial cell dysfunction observed in the current study.

Endothelial regulation in endothelial cell dysfunction. However, whereas neutrophil-mediated endothelial cell dysfunction is well documented, endothelial cell dysfunction also can occur in the absence of blood cells, suggesting that endothelial-produced factors contribute to endothelial cell dysfunction (52, 53). Whereas the presence of NHE-1 on the basolateral surface of endothelial cells has been demonstrated (7, 41), to date, the role of NHE in endothelial cells has not been elucidated. Several studies have examined in vitro the role of NHE in endothelial cell pH response to changes in shear stress (50, 51). An increase in fluid shear stress produces a decrease in intracellular pH (pH_i) within seconds that appears to be due to activation of an alkali extruder and the NHE (51), whereas a decrease in shear stress results in an increase in pH_i via Na^+-dependent Cl^--HCO_3^- exchange and NHE (50). Inhibition of NHE with HOE-694 elicited intracellular acidification and inhibited NO release under conditions of altering shear stress (1). Similarly, pharmacological inhibition of endothelial NHE with HOE-694 also significantly blocked the production of NO after stimulation with bradykinin, suggesting a role of the exchanger or endothelial pH_i in determining the activity of agonist-induced, endothelium-dependent vasodilatory function (12). The activity of constitutive NO synthase in the endothelium is tightly controlled with small changes in pH, specifically a decrease in pH_i, inhibiting the enzyme (12). Thus it would appear that NHE-1 and other pH_i-regulating mechanisms play a role in altering NO synthase activity and therefore NO production. As stated earlier, it is the endothelial cell-dependent production of NO that is involved in the vasodilatory response to acetylcholine (37, 40). Inhibition of NHE activity may also reduce endothelial synthesis or release of vasodilatory prostaglandins, because the stimulation of the exchanger appears to mediate agonist-induced prostaglandin release (14). Thus from these data one might expect NHE inhibition to actually attenuate the endothelial-dependent vasodilatory response, because acetylcholine has been reported to induce endothelium-dependent relaxation by the generation of NO, prostaglandins, and leukotrines through NO synthase, cyclooxygenase, and lipooxygenase pathways, respectively (36). However, the referenced experiments were carried out on isolated endothelial cell cultures not grown under shear stress conditions, thus whether this response occurs in vivo is not clear. Our study suggests that in vivo a more complex series of events/interactions occurs and that inhibition of NHE during ischemia-reperfusion protects endothelial cell function.

Alternatively, previous studies suggest that impaired endothelium-dependent relaxation in hypertension and atherosclerosis is due neither to decreased NO synthase activity nor to a deficiency in L-arginine availability but rather to an accelerated inactivation of NO by superoxide radical (12). Previous work shows reoxygenated endothelial cells are potent generators of oxygen radicals at concentrations sufficient to induce cell injury and death (53). H_2O_2 also has been shown to induce calcium oscillations in endothelial cells (23). Furthermore, treatment of cultured human aortic endothelial cells with H_2O_2 caused a decrease in endothelial cell pH_i that was attenuated by pretreatment with ethylisopropylamiloride (23, 24). Finally, hypoxia/reoxygenation of human umbilical vein endothelial cells induced endothelial cell dysfunction as measured by fura 2 but not cell death as measured by ^{51}Cr leak (13). This reaction was thought to occur because calcium ions were abruptly transferred due to membrane damage induced by hypoxia/reoxygenation (13, 39) secondary to rapid production of oxygen radicals produced during reperfusion of cultured hypoxic endothelial cells (53). In fact, confocal imaging of endothelial cells subjected to hypoxic injury revealed localized increases of intracellular calcium at sites of bleb formation (25), suggesting that increased intracellular calcium contributes to endothelial cell damage during hypoxia. Whereas the effects of calcium overload have been minimally studied in endothelial cells in cardiomyocytes, NHE inactivation has been shown to inhibit calcium overload and the subsequent detrimental effects (22, 34, 44).

Endothelin in endothelial cell dysfunction. Elevated plasma endothelin levels have been demonstrated in patients with coronary artery disease (30) and acute coronary syndromes (35, 49). In myocardial tissue endothelin-1 is known to activate NHE in myocardial tissue resulting in intracellular alkalinization (28) and increased myofibril sensitivity to calcium (48). In the coronary vasculature, endothelin at pathophysiological concentrations has been shown to mediate coronary vasoconstriction in the dog (6). Furthermore, endothelin-1 was shown to contribute to abnormal endothelium-dependent relaxation after ischemia-reperfusion injury in rabbits hearts (20) possibly due to impaired efficacy of endogenous NO production (4). Finally, administration of HOE-642 increases baseline coronary flow and prevented endothelin-1-induced reduction in coronary flow in isolated perfused rat hearts (5). Thus NHE-1 inhibition may counteract the vasoconstrictive effects of endothelin following myocardial infarction.

In conclusion, this study demonstrates for the first time that NHE-1 inhibition significantly attenuates endothelial cell dysfunction induced by prolonged myocardial ischemia-reperfusion injury. However, because the current study did not specifically address mechanisms, it is not clear whether NHE-1 inhibition reduces endothelial cell dysfunction by attenuating neutrophil-mediated damage to the endothelium or by directly protecting the endothelium. Future studies will focus on dissecting the mechanism by which NHE-1 inhibition preserves endothelial cell function following ischemia-reperfusion injury.

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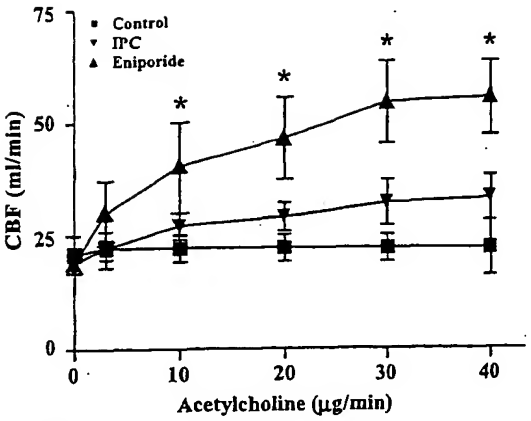

Fig. 3. Effect of NHE-1 inhibition on coronary blood flow (CBF) following ischemia-reperfsuion injury. IPC (1 × 5′), eniporide (3.0 mg/kg), or saline (CON) was administered 15 min before occlusion of LAD for 90 min. Data are expressed as mean CBF (ml/min) vs. acetylcholine infusion dose. n = 9 (CON), 8 (IPC), and 7 (eniporide) dogs. *$P < 0.05$ vs. control group.

contrast, treatment with eniporide significantly preserved endothelial cell function (Fig. 3).

DISCUSSION

The effects of NHE-1 inhibition in a prolonged ischemia model were examined for several reasons. NHE-1 inhibition has been shown to be more efficacious than IPC at conferring cardioprotection against prolonged myocardial ischemia (17). Thus we examined whether similar protective effects occurred with regard to endothelial cell function. When ischemic time is relatively short, endothelial and myocardial dysfunction appear to follow a similar time course (31). However, prolonged ischemia leads to more severe endothelial dysfunction compared with myocardial dysfunction (31). Furthermore, morphological evidence of coronary vascular injury appears to be a late sequelae of ischemic injury because ultrastructural evidence of vascular injury is seen only after prolonged ischemia (27). Thus we postulated that a prolonged ischemic insult would produce severe irreversible endothelial dysfunction independent of the myocardial damage (or protection) observed in control, IPC-, or NHE-1 inhibitor-treated animals.

Our data demonstrate that, whereas an ischemic insult of 90 min followed by 180 min of reperfusion abolishes the coronary vasodilatory response to acetylcholine in control and IPC-treated animals, comparable to previous reports (2), inhibition of the NHE-1 with eniporide significantly preserves the endothelial-mediated vasodilatory response to acetylcholine (Fig. 3). These data demonstrate for the first time that inhibition of NHE-1, in addition to its cardioprotective effects, prevents ischemia-reperfusion-induced endothelial cell dysfunction.

Infarct size and vasodilatory reserve. Since 1982, when the phenomenon of postischemia-reperfusion endothelial cell dysfunction was first described in dogs (29), a number of studies have demonstrated that ischemia (15–90 min) followed by reperfusion (30–90 min) results in endothelial cell dysfunction both in isolated arterial rings (21, 45, 46) and in vivo (8, 26, 33, 38). Subsequent studies have determined that endothelial cell-dependent production of nitric oxide (NO) is involved in this response (37, 40). The time course of ischemia-reperfusion-induced endothelial cell dysfunction has varied depending on the species examined (21, 43, 45). In humans, reduced coronary vasodilator response has been demonstrated after myocardial infarction. Despite successful recanalization of the infarct artery by thrombolysis, after an acute myocardial infarction the vasodilatory response in the infarcted myocardial region remains severely impaired. In dogs, the vasodilator response in regions of viable myocardium is preserved at the onset of reperfusion (2, 26, 47). In contrast, in the necrotic myocardium the loss of vasodilator reserve is apparent at the time of reperfusion (47). However, in the dog in vivo a normal vasodilatory response has been observed during the initial 30-min following reperfusion with deterioration during the subsequent hours (2, 47). In a canine model of myocardial ischemia-reperfusion injury employing 90 min of ischemia, it was demonstrated that a significant proportion of myocytes within the infarct region are viable at the time of reperfusion and lose viability during reperfusion, suggesting specific cell death due to reperfusion injury (32). Previous studies from our laboratory (17) suggest that NHE inhibition may protect myocardial cells within the AAR that otherwise may have been irreversibly injured on reperfusion (17). As shown in the current study, treatment with eniporide conveys significant cardioprotection from a prolonged ischemic insult as measured not only by histochemical infarct size (Fig. 1) but also by more sensitive markers of cardiomyocyte damage such as CK and troponin I (Fig. 2). Therefore, the vasodilator response may reflect the relative amount of viable and necrotic myocardium in the ischemic-reperfused territory, and progressive loss of vasodilator reserve may represent progression to necrosis.

Endothelial dysfunction and neutrophils. However, consistent with the time course of endothelial cell dysfunction is the fact that following myocardial ischemia-reperfusion injury, neutrophils accumulate in the ischemic myocardium where they contribute to myocardial damage via release of superoxide radicals (9–11). It is well established that the interaction between polymorphonuclear neutrophils (PMN) and endothelial cells at reperfusion leads to endothelial cell dysfunction (26, 38, 43). A number of studies demonstrate a reduction in myocardial infarction and a preservation of endothelial cell function via administration of agents that inhibit neutrophil activation and/or influx into the ischemic-reperfused myocardium (19). More recently, we demonstrated that NHE-1 inhibition also might reduce PMN-mediated myocardial damage that occurs following reperfusion (16). NHE-1 inhibition attenuated platelet-activating factor-mediated PMN respiratory burst and decreased PMN accumulation within the

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